Effects of Inhaled Acids on Airway Mucus and Its Consequences for Health

by Bo Holma*

The high molecular fractions, i.e., > 100,000 dalton, are found to be most responsible for the H^+ ion absorption capacity of the mucus in the respiratory tract. This function serves as a protection against the penetration of the H^+ ion to the surrounding tissue. Acidifying mucus with a high concentration of protein, mainly glycoproteins, results in increased viscosity, which affects various lung functions. After acid saturation of the mucus, the H^+ ion will react with the epithelial tissue, which results in increased permeability and a variety of effects.

Acidic mucus or mucus with a low protein concentration, as in some asthmatics, constitutes a base for risk groups regarding acidic exposures. A rough estimate indicates that persons with normal mucus buffer capacity and protein content can tolerate about 3000 µg SO_2/m^3 or 300 µg H_2SO_4/m^3 per 30 min.

Introduction

Acidic pollutants have long been claimed to have deleterious effects on different lung functions. Especially, sulfur dioxide (SO_2) and its derivatives have been investigated most intensively. Epidemiological studies have demonstrated correlations with these substances and impaired lung functions, particularly in infants, infirm people, and people with pulmonary diseases. The substances mentioned have also been found to be correlated with increased mortality, e.g., in Denora, PA, in 1948, and in London in 1952. In spite of the fact that epidemiological studies are unable to uncover one causal factor from all the other factors present in air pollution situations, these studies have constituted the base for standard settings of air quality by governmental and international agencies. However, more attention should be given to experimental work, as it is performed under more well-defined and controlled conditions, regarding concentration, exposure time, lung functions, and histochemical and biochemical changes, for example. Experimental studies can also contribute to the understanding of the mechanisms behind the pathophysiological changes and to the estimation of critical concentrations for different groups of people.

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Effect Dependence of Mucus pH, Buffer Capacity, and Protein Concentration

From experimental studies it is known that exposure to acidic pollutants does increase airway resistance, especially in asthmatics. Some of these studies have demonstrated this increase at exposure to the lowest acidic concentration tested (1,2). There are indications that increased airway resistance is due to reduced buffer and H^+ ion absorption capacity of airway mucus (3,4), which is mainly a function of the high molecular weight mucus proteins (Fig. 1), especially the glycoproteins (4). A decreased capacity to protect the H^+ ion penetration of surrounding tissues will thus be found in mucus with a low concentration of high molecular weight proteins or a low buffer capacity (Fig. 2). The same effect is observed for acid mucus (Fig. 3). For mucus with nearly acid-saturated proteins, any excess of H^+ ion over what can be neutralized by the ammonia in the respiratory tract (5) will gain access to the surrounding tissues and initiate reactions.

Intra- and intercellular edema begin to occur at pH values below approximately 6.5 in the mucosa of cow trachea exposed for 20 hr to sulfuric acid (H_2SO_4) solutions *in vitro*, and, below pH 6, the epithelial cells loosen from each other and the basement membrane (6). Also, *in vivo* the same effects have been found to
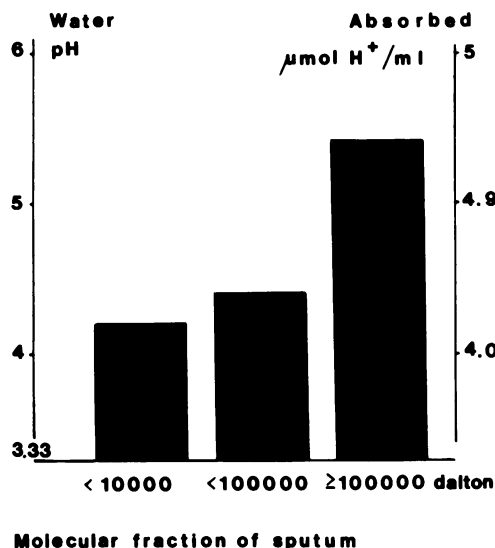
FIGURE 1. H+ ion absorption capacity of three different molecular-sized fractions of sputum in physiological salt solution of pH 3.33.

constitute the earliest histopathological changes in the noses of mice exposed for 24 hr to SO_2, 28,000 $\mu g/m^3$ (7) and in dogs after 1 year exposure to SO_2, 2800 $\mu g/m^3$ (8). In the latter study, a reduction of the tracheal mucus velocity was also noticed. In other studies acidic exposure was also found to reduce the velocity of the mucus transport (8–11).

Effects on Mucus Rheology and Transport

The viscoelastic properties of the mucus secretion are fundamental for mucus transport (12–19). The maximum rate of transport decreases with increased or decreased viscoelasticity (12,13,16,17,19–22) and/or nondialyzable solids (19).
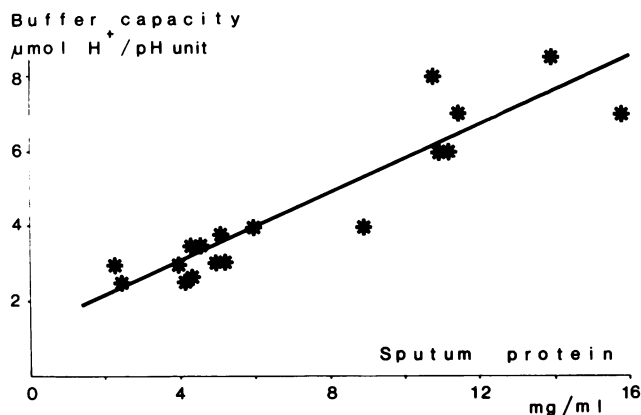

FIGURE 2. The correlation (least-square method) between the total protein (Lowry-albumin standard in sputum and its buffer capacity, expressed as the number of μmoles of H+ ion required to decrease the pH in sputum one full unit. The sputa were collected from six smokers. $r = 0.93$; $y = 0.46x + 1.23$.
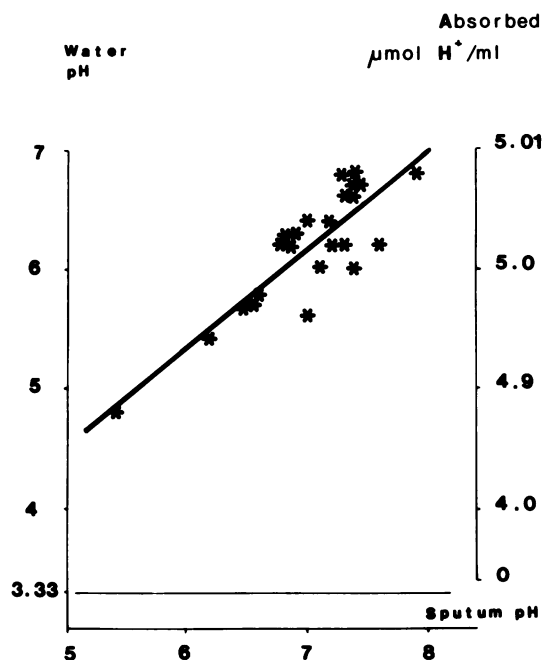

FIGURE 3. The H+ ion absorption of 1 mL native sputum with different pH values in 10 mL acidified water. The sputa were collected from six smokers. $r = 0.85$; $y = 0.77x + 0.78$.

The H+ ions absorbed in the mucus increase its viscosity (3,4), which also correlates with the mucus content of acids (14,23–28). The viscosity of the mucus is further dependent on the concentration of proteins (Fig. 4), mainly the glycoproteins (13,17,18,27,29–34); ionic composition and strength (4,21); and the pH value (3,13,14,18,29,35,36). The viscosity can further be increased by airway infections (37), e.g., due to increased content of lysozyme, derived mostly from macrophages (29). The importance of the glycoproteins in the mucus protection of the surrounding tissues is also illustrated by the existence of lectins on the surface of bacteria with combining sites complementary to the oligosacharides linked to the protein core of the glycoprotein (38).

The viscosity of the glycoproteins increases with increased acidity. Under certain conditions, the viscosity also increases under alkaline treatment. This function seems to be determined by the protein concentration and varies further with the ionic strength (4,39).

The pattern of the isomaxima lines for the dynamic viscosity of the glycoproteins as a function of the pH, and the ionic strength corresponds to the gel status of some proteins, e.g., serum albumin and β-lactoglobulin (39). This status is critically balanced between the composition and concentration of the proteins, electrical charged groups, the order of entanglements of the molecules, the pH, and the ionic composition and strength. This is illustrated by the fact that a Ca^{2+} ion concentration of 3.8 mM in combination with 85 mM NaCl can convert protein gels to sols (39). Both the storage modulus (G') and the transport rate of the
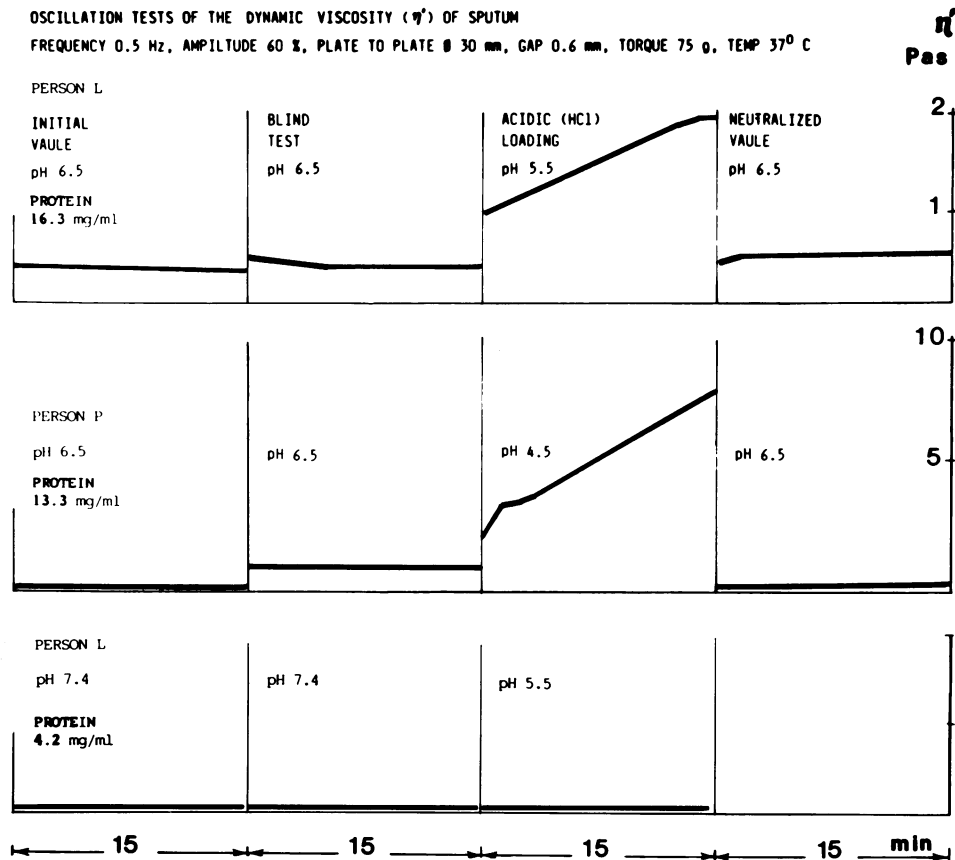
OSCILLATION TESTS OF THE DYNAMIC VISCOSITY (η') OF SPUTUM
FREQUENCY 0.5 Hz, AMPILTUDE 60 %, PLATE TO PLATE ∅ 30 mm, GAP 0.6 mm, TORQUE 75 g, TEMP 37° C

η'
Pas

PERSON L

INITIAL	BLIND	ACIDIC (HCl)	NEUTRALIZED
VAULE	TEST	LOADING	VAULE
pH 6.5	pH 6.5	pH 5.5	pH 6.5
PROTEIN			
16.3 mg/ml			

PERSON P
pH 6.5   pH 6.5   pH 4.5   pH 6.5
PROTEIN
13.3 mg/ml

PERSON L
pH 7.4   pH 7.4   pH 5.5
PROTEIN
4.2 mg/ml

├───── 15 ─────┼───── 15 ─────┼───── 15 ─────┼───── 15 ── min ─┤

FIGURE 4. The dynamic viscosity (n') in sputa with different concentration of proteins before and after being acidified with HCl, *in vitro*. The n' increased only in the sputa with the higher content of proteins after the addition of the acid and reversed to normal again after neutralization with NaOH.

mucus have been found to have a maximum for divalent ion around 0.5 mM (*21*).

Contribution of Acidified Mucus on Airway Resistance

In addition to the effect on mucus transport, increased mucus viscosity has been demonstrated to correlate with increased airway resistance (*25,26,29,40–42*) and reduced pulmonary gas exchange (*43*). This might be of special concern for smokers, who have been found to have an increased concentration of lysozyme, derived from an increased number of macrophages (*24,29*). There are indications that lysozyme can crosslink the glycoproteins (*24,29*).

No simple explanation of the bronchoconstriction due to acidic exposure exists. The increased epithelial permeability of chemicals into receptors due to increased acidity of the mucosa (*6*) might facilitate different reactions from the surrounding tissues. For example, the mast cells have an optimum for histamine release at pH 6.8 to 7.1, which decreases sharply on the acid side (*44,45*) and reduces the possibility of histamine-produced bronchoconstriction at lower pH values in the mucosa.

The decrease of the SO_2-induced airway resistance after atropine administration does not prove that this resistance is caused exclusively by a parasympathetic stimulus. This statement is based on the fact that atropine is both an acetylcholine antagonist and an inhibitor of the secretory glands, i.e., for the production of glycoproteins. At the same time, the sol phase of the mucus is kept at an optimal level by the goblet cells and direct passage of fluids from the capillaries between the epithelial cells (*46,47*) and the intercellular fluids, especially at low pH of the mucosa (*6*). The effect of mucus volume and its physical properties also changes, with consequences in the form of lower airway resistance (*26,27,40–42,48*). Further denervation of the lung has been reported to reduce the viscidity of sputum (*49,50*), which is correlated with decreased airway obstruction (*26,27,40–42*).

The increased airway resistance demonstrated in some SO_2 exposures after administration of parasympathomimetic drugs (*51*) might be explained by an increased depth of the mucus and closer contact between the glycoproteins and other components of the mucus. The narrowing of the bronchiolar cross-section produced by these drugs could rapidly increase the thickness of the fluid layer (*52*), thus favoring gel formation and the viscoelastic properties of the mucus.

Such changes are further increased by acidity (*3,4,14,23,25–28,53*).

Risk Groups

The effects of acidic exposure might be released by reactions from the H^+ ion absorption in the mucus and/or, after penetration of this barrier, from the surrounding tissues. The first type of reactions based on changes in the mucus rheology has not been observed to occur unless the mucus protein concentration exceeds about 6 mg/mL (*4*). As concentrations over this level are unusual even in smokers (*4*), it may be unexpected in normal, nonsmoking individuals. The reactions to be expected in normal individuals should mainly be released from the surrounding tissues. The determining factor for this is the H^+ ion absorption capacity and buffer characteristics of the mucus. Data about this does not exist for mucus from healthy persons. Hence, only values from abnormal sputum are available for judging the threshold values for acidic pollutants for normal people. On the other hand, it might be of more concern to establish the levels acceptable for different risk groups. The group of primary interest is those asthmatics and others with mucus of extremely low pH. For those with mucus pH values under the critical value (around pH 6.5), one can expect detrimental effects in their respiratory epithelium even under normal conditions, i.e., without the addition of acidic pollutants. This suggestion is supported by a higher concentration of serum transudate in the mucus of some asthmatics (*54,55*). Their acidic mucus might not only explain an increased epithelial permeability (*6*) but also the complete shedding of the epithelium found in patients dying from an attack of asthma (*28*). For this group of people it is unrealistic to establish a threshold level under which no adverse effects can occur.

Another risk group is smokers with increased lysozyme and total protein in their mucus. In this kind of mucus with high protein content, the H^+ ions are absorbed first by the proteins, until saturation producing an optimum of mucus viscosity is reached (*3,4*). Thereafter, the acidic exposure will be more pronounced for the surrounding tissues, with increased epithelial permeability facilitating a variety of reactions. A rough estimate indicates that this state can be expected to occur for smokers somewhere between 3000 to 6000 µg SO_2/m^3, or about 300 µg H_2SO_4/m^3 (30-min values) (*3*). The initial result would be a reduced mucus transport velocity and/or increased airway resistance, disappearing about 30 min after completion of the exposure, depending on the transit time of the mucus in different parts of the respiratory tract. These levels of acidic pollutants are indicated to be the lowest levels also for nonsmokers (*9,10*) and thus no effect is to be expected in normal adults below those demonstrated in experimental research (*3*).

As the composition of the mucus protein determines the pH-dependent rheology of the mucus, different diseases can constitute risk groups (*27,55*). The same might be true for neonates, whose mucus is not fully developed until some months after birth (*30*).

Recommendation

To facilitate the risk estimation of health effects due to acidic pollutants, especially combined effects, the air pollution measurement should contain data on the moles of H^+ ion/per cubic meter of air that can be released by reaction with water.

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